IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: DOMAZAKIS, Emmanouil	:	Examiner: STULII, Vera
Serial No.: 10/577,659	:	Group Art Unit: 1781
Filed: May 1, 2006	:	Attorney Docket No.: 506845.3
For: Method of production of meat products from entire muscular tissue, with direct incorporation of olive oil	:	Customer No.: 27526 Confirmation No.: 8474

Via EFS-Web

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF GEORGE STEPHANOPOULOS PURSUANT TO 37 C.F.R. § 1.132

1. I am currently the A. D. Little Professor of Chemical Engineering at the Massachusetts Institute of Technology. My PhD is in Chemical Engineering and I have been in this position for 27 ½ years and have been involved with teaching, research, technology development, and industrial consulting with more than 50 companies in food processing, chemicals, pharmaceuticals, etc. My expertise is in process engineering and I have been involved with a very broad variety of process-product combinations in the food industry and the other industrial sectors mentioned above. I have also worked as Chief Technology Officer for the Group of companies of Mitsubishi Chemical Corporation in Tokyo, Japan, where for 5 years I was in charge of R&D and technology for new business. In this capacity I was the Managing Officer responsible for the Intellectual Property Department of the Corporation and was responsible for Patent Strategy and Patent Defense.

2. I am an author or co-author of many publications. These include:

A. Authored-Coauthored Books

1. "Synthesis of Heat Exchanger Networks," in *Industrial Energy Conservation*, E. Gyftopoulos (Series Editor), MIT Press (1982).

2. *Chemical Process Control: An Introduction to Theory and Practice*, Prentice-Hall (1984). Also in Greek and Chinese translations

3. *Solutions Manual; Chemical Process Control: An Introduction to Theory and Practice*, Prentice-Hall (1985).

4. *Analysis & Planning of Greek Petrochemical Industry*, KEPE, Athens (1986).

5. *The Scope of Artificial Intelligence in Process Engineering*, CACHE Monoghraph (1990).

6. *Intelligent Systems in Process Engineering: Paradigms for Product and Process Design*, by George Stephanopoulos and Chonghun Han, Volume 21 in the "*Advances in Chemical Engineering Series*", Academic Press (1995).

7. *Intelligent Systems in Process Engineering: Paradigms for Process Operations and Control*, by George Stephanopoulos and Chonghun Han, Volume 22 in the "*Advances in Chemical Engineering Series*", Academic Press (1995).

B. Edited-Coedited Books

1. "*Artificial Intelligence in Chemical Engineering Research and Development*" (Geo. Stephanopoulos and M. Mavrovouniotis, Editors), Special Issue of *Computers and Chemical Engineering*, Pergamon Press (1988).

2. *CACHE Case-Studies Series in "Knowledge-Based Systems in Process Engineering"*, 3 Volumes. CACHE (1988).

3. *CACHE Monograph Series in "Artificial Intelligence in Process Engineering"*, edited with J. Davis, 3 Volumes published, 2 in preparation. CACHE (1990).

4. *Foundations of Computer Aided Process Design*, J. J. Siirola, I. E. Grossmann and Geo. Stephanopoulos (editors), CACHE-Elsevier (1990).

5. *On-Line Fault Detection and Supervision in the Chemical Process Indistries*, P.S. Dhurjati and Geo. Stephanopoulos, *IFAC Symposia Series*, No.1 (1993)

6. *ISPE '95: Intelligent Systems in Process Engineering*, Geo. Stephanopoulos, J.F. Davis, and V. Venkatasubramanian (editors), *AIChE Symposium Series*, Vol. 92 (1996)

7. *Proceedings of the European Symposium on Computer-Aided Process Engineering, ESCAPE-6*, Volumes 1 and 2, Geo. Stephanopoulos (editor), *Computers and Chemical Engineering*, (May 1996)

8. *Selected Papers- ESCAPE-6*, Special Issue of *Computers and Chemical Engineering*, Geo. Stephanopoulos and E. Kondili (editors) (1998)

9. *IFAC Proceedings: Dynamics and Control of Process Systems-2001*; Geo. Stephanopoulos, J.H. Lee, and En Sup Yoon, editors. Pergamon Press, 2001.

C. Papers Published in Refereed Scientific Journals: 214

D. Papers Published in Conference Proceedings: 185

3. This Declaration is being presented by me in furtherance of the prosecution of the above-referenced application.

4. I have reviewed the above-referenced application in detail as well as Domazakis (U.S. Pub. No. 2003/0049364), Brandt (Marinades "Meat" Challenge publication) and Hendricks et al. (U.S. Pat. No. 5,053,237), which have been cited during prosecution. I have compared the

method presented in the cited references to the method of the invention disclosed and now claimed in the present application, herein referred to as "App. 10/577,659." After reviewing these references, it is my firm conviction that these references do not render the claimed invention obvious.

5. Although vegetable oil-containing meat products of emulsion-type, may be retrieved in the literature (Dubanchet, U.S. Pat. No. 5,238,701; Bloukas & Paneras[1], 1993, attached hereto as Exhibit A), no evidence has been provided so far with regards to processed, ready-to-eat meat products based on entire-muscular tissue, wherein olive oil has been stably incorporated. This, by no means, indicates a lack of interest in the development of such products, but rather confirms the technological difficulties implicated in the making of these types of products. Instability in the incorporation of oil is indeed expected to result in the phenomena addressed by the Applicant in page 1, lines 32-44 of App. 10/577,659. The claimed invention has thus addressed a long-felt need in the industry and succeeded to achieve this goal.

6. There is nothing in the cited references themselves or in the knowledge generally available to a person of ordinary skill in the art, at the time App. 10/577,659 was filed, that would lead one of ordinary skill in the art to combine the cited prior art. First of all, the only prior art that at least indicates combination of entire muscular tissue and vegetable oils is Hendricks, yet the goal of the invention, the method followed and the products resulting therefrom, have nothing to do with the goal, the claimed method and resulting products of the present application. Clearly, the goal in Hendricks is to upgrade the tenderness and sensory qualities of fresh red meats, thus improving their market value. However, the deposition of oil inside the mass of a fresh raw meat, by means of an injection apparatus, is substantially different

[1] J. G. Bloukas & E.D. Paneras. *Substituting olive oil for pork backfat affects quality of low-fat frankfurters*, Journal of Food Science, vol. 58 (4), 1993

to the stable oil incorporation, as achieved by the method described in the present patent, in a sliceable ready-to-eat meat product based on entire-muscular tissue. In the latter case, the mechanical working (=tumbling), as well as the presence of sodium chloride, have led to the extraction and solubilisation of myofibrillar proteins, which, surprisingly, were found capable of forming a stable composition on the surface of the meat pieces with the added oil and the free water (by means of emulsification and/or entrapment phenomena). That was an interesting and surprising effect. It is, therefore, the precise localization of the stably dispersed oil droplets, that characterizes the uniqueness of the product resulting from the present application. The novel aspect of App. 10/577,659 is reflected in the description of the critical process features, which allowed for the stable incorporation of the oil droplets in the precise location. In my opinion, neither the precise localization of the dispersed oil globules, nor the critical process features which contributed to the novel aspects of this invention, may be derived from the cited prior art, even if this is considered by the combination of the different references.

7. Hendricks relates to injected pieces of fresh raw meat, which is intended for home cooking. Hendricks merely discloses the use of an "injectate", which is disclosed as a composition that penetrates, by means of pressure injection, the muscular tissue, obviously at an injection depth. Retainment of the delivered injectate, comprising oil, within the muscular tissue was rather challenged, due to the non-stable incorporation of the injectate within the meat mass. The addition of a binder in the composition improved the retention of the injectate. It is thus evident that the physicochemical mechanisms that underline the oil incorporation in the *cooked processed product* of App. 10/577,659, are nowhere disclosed, nor even indicated in Hendricks. The function of "activated" myofibrillar proteins at the surface of meat pieces, which is of primary significance in the mechanism of oil incorporation in App. 10/577,659, is absent in

Hendricks. Rather, Hendricks uses added ingredients, such as non-meat ingredients (e.g. methyl cellulose) to retain the injectate *within* the meat mass. Moreover, the characteristic localization of the dispersed oil phase, as well as the critical process features that ensure the stable incorporation thereof, in the cooked processed product, could not be derived by Hendricks. In my opinion, Hendricks would not even been considered by a person skilled in the art, dealing with the making of *processed ready-to-eat* entire muscular tissue-based cooked products. Moreover, to the extent of my knowledge, I do not recall having seen products resulting from the patented method of Hendricks.

8. In my opinion it would not make sense to one skilled in the art to combine any of the remaining prior art with Domazakis since Domazakis describes the admixture of oil in a finely comminuted meat paste, along with other added ingredients (e.g. phosphates, non-meat proteins and starch) and Brandt describes some basic technological issues regarding marinating fresh meat pieces, such as the use and composition of a marinating solution. Brandt refers to products, such as the Hatfield Marinated Fresh Pork, which are made by injecting a 10% solution, followed by massaging and vacuum packaging (Brandt, page 6 of 7). In fact, Brandt teaches away from the addition of a "non-soluble to water" ingredient, if his instructions should be considered (Page 2 out of 7, 3rd paragraph: "All of the ingredients should be dispersed in ambient temperature water for proper dissolution.") Therefore, Brandt does not teach anything about a fatty substance, let alone olive oil.

9. To my opinion, the cited prior art, either examined individually or in combination, does not provide the critical technical features of the claimed method of App. 10/577,659, including (i) *adding olive oil* to the fully *tumbled and brine-injected entire muscular tissue*, and (ii) proceeding to a second independent *tumbling step after the addition of olive oil.*

10. Accordingly, it is my opinion that the present invention is unique and not obvious based upon my experience in the industry, in view of the unsolved and long-felt need in the industry, and the cited references.

11. I declare that all statements made herein are of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and such willful, false statements may jeopardize the validity of any patents issued from the patent application.

June 17, 2011
Date

George Stephanopoulos
George Stephanopoulos

EXHIBIT A

Substituting Olive Oil for Pork Backfat Affects Quality of Low-Fat Frankfurters

J.G. BLOUKAS and E.D. PANERAS

ABSTRACT

Low-fat frankfurters (10% fat), formulated for 10%, 12% and 14% protein, were made with olive oil. Compared to control (27.6% all animal fat, 10.9% protein) they had similar flavor, lower ($P<0.05$) TBA values and reduced (44.7–47.6%) caloric content, but had lower ($P<0.05$) processing yield (5.5–6.5%) and overall palatability. Among low-fat treatments, samples with 12% protein had better-quality characteristics. The 12% protein frankfurters compared to the control (except for palatability), had similar ($P>0.05$) sensory attributes and higher ($P<0.05$) skin strength and improved texture. The treatment with 10% protein had undesirable color and was very soft. That with 14% protein had the same ($P>0.05$) red color as the control but higher ($P<0.05$) firmness, skin strength and textural traits and lower ($P<0.05$) juiciness.

Key Words: olive oil, frankfurters, fat substitution, low fat, meat products

INTRODUCTION

IN MOST industrialized societies consumers are recommended to reduce energy intake and to reduce fat intake to 30% or less of total caloric intake (AHA, 1986). Manufacturing calorie-reduced foods, which include low-fat meat products, is of both economic and health interest (Wirth, 1988). Frankfurter type sausages produced with pork fat have up to 30% fat. Pork fat has about 40% saturated fatty acids (Briggs and Schweigert, 1990) while cholesterol is the most important sterol present.

Saturated fat is considered a primary cause of hypercholesterolemia (Mattson and Grundy, 1985) and oxidation products of cholesterol also have adverse human health effects (Pearson et al., 1983; Addis, 1986; Maerker, 1987). Although polyunsaturated fatty acids decrease plasma LDL-cholesterol (Mattson and Grundy, 1985), they promote carcinogenesis in experimental animals (Clinton et al., 1984). In contrast to saturated and polyunsaturated fats, diets high in monounsaturated fat have been associated with decreases in coronary heart disease. Prevalence of heart disease was relatively low in areas of the Mediterranean region in which diets high in monounsaturated fat are typically consumed (Keys, 1970; Keys et al., 1986; Aravanis and Dontas, 1978). Thus incorporation of monounsaturated fats in meat products may have a positive effect on consumer health.

St. John et al. (1986) increased the monounsaturated/saturated fatty acid ratio in low-fat frankfurters using the lean and fat from pigs fed elevated levels of canola oil which contains 64% oleic acid. Shackelford et al. (1991) studied the acceptability of low-fat frankfurters as influenced by feeding of elevated levels of monounsaturated fats to growing-finishing swine. They reported that the high-oleate treatments were comparable to the control in all sensory characteristics. Rlendeau (1990) incorporated canola oil into smoked sausages and found that fat and calorie-reduced products were acceptable in quality. Park et al. (1989, 1990) studied the properties of low-fat frankfurters manufactured by direct incorporation of high-oleic sunflower oil (HOSO) as a source of monounsaturated fat. They reported that low-fat frankfurters with maximum allowable added water and HOSO could be manufactured without adverse effects on processing yield, texture or sensory properties.

Virgin olive oil is the most monounsaturated vegetable oil. It contains 56.3–86.5% monounsaturated fatty acids, 8–25% saturated and 3.6–21.5% polyunsaturated fatty acids (IOOC, 1984). It also has tocopherols and phenolic substances which act as antioxidants. Olive oil has a high biological value attributed to its high ratio of vitamin B to polyunsaturated fatty acids (Viola, 1970). It also has a lower ratio of saturated to monounsaturated fatty acids and the presence of antioxidant substances at an optimum concentration (Christakis et al., 1980).

Our objectives were to evaluate quality of low-fat frankfurters (<10% fat) produced by direct incorporation of virgin olive oil as a sole source of monounsaturated fat, and to study effects of protein level in the finished product on quality characteristics.

MATERIALS & METHODS

Ingredients and formulation

Commercial frozen beef meat, fresh pork meat and pork backfat were obtained from the local meat market. Partially thawed beef and the fresh pork were trimmed of separable fat to provide extra lean meats. The lean meat and the pork backfat were separately ground through a 12 mm plate and then through a 3 mm plate. The ground meats and pork backfat were vacuum packaged and frozen at −20°C for 1–2 wk until product formulation. Representative samples were analyzed for moisture, fat and protein (AOAC, 1984) prior to freezing. All raw materials were tempered at 0°C for 24 h prior to use.

Virgin commercial olive oil containing 0.71% free fatty acids (as oleic) was pre-emulsified the day of use. Eight parts of hot water were mixed for 2 min with one part sodium caseinate. The mixture was emulsified with 10 parts oil for 3 min (Hoogenkamp, 1989a, b).

Four treatments were prepared (Table 1). The control was produced using only pork back fat formulated to 28% fat and 11% protein. These values represent about the mean fat and protein content of commercial frankfurters in Greece (Bloukas and Paneras, 1986). The

Table 1—Formulation Ingredients

Ingredients (g)	Control[a] A	Low-fat treatments[b] B	C	D
Protein (%)	11	10	12	14
Beef lean (1.32% fat)	700	830	1020	1200
Pork lean (3.87% fat)	1000	1170	1430	1700
Pork backfat (76.84% fat)	1700	—	—	—
Olive oil[c]	—	415	405	395
Ice / water[d]	1630	2615	2175	1735
Sodium chloride	85	87	87	87
Sodium nitrite	1	1.2	1.2	1.2
Sodium ascorbate	3	4	4	4
Phosphates	12	12	12	12
Sodium caseinate	50	50	50	50
Starch	200	200	200	200
Seasoning	24	32	32	32

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein.
[c] Percent in batter composition: 7.8%, 7.4% and 7.2%, respectively.
[d] Percent in batter composition: 30.9%, 48.2%, 40.1% and 32.0%, respectively.

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other three treatments were produced with olive oil formulated to give a final product with less than 10% fat and 10%, 12% and 14% protein, respectively. In low-fat treatments the added salt was reduced while the amount of seasonings was increased as suggested by Wirth (1988, 1991) and Hoogenkamp (1989b). All treatments were replicated three times from separate meat and fat sources at three different time periods.

Frankfurter manufacture

The partially thawed lean was mixed with curing ingredients and dry chopped for 20–30 sec in a Laska 30L cutter at low speed. After dry chopping about half the water was added in the form of ice and the chopping continued until a temperature of +3°C was reached. At that point the thawed pork backfat, pre-emulsified olive oil, seasoning and other ingredients, together with the remainder of the ice/water, were added and the batter was chopped at high speed until the final temperature reached 12°C.

Immediately after chopping the batter of each treatment was vacuum stuffed into 24 mm diameter Nojax cellulose casings. Each treatment was handlinked at 15 cm intervals and the frankfurters were heat processed and smoked in a smokehouse to internal temperature 72°C (Hoogenkamp 1989a and b, Wirth 1988, 1991). The frankfurters were showered for 15 min and chilled at +2°C for 24 hr. After chilling the frankfurters were peeled, vacuum packaged (vacuum level 650 mmHg) in film pouches with a reported oxygen permeability rate of ≈116cm³/m²/24 hr/1 atm (23°C, 0% RH) and stored in the dark in a cooler at +4°C until subsequent analysis.

Batter properties

Immediately after processing the following parameters of batters were determined: pH was determined with a WTW digital pH meter with corrections for temperature differences. Viscosity was measured immediately after batter preparation with a Brookfield digital viscometer, model DV-II, set at 2.5 rpm and equipped with a spindle No 5. Frankfurters were weighed before heat processing and smoking and after chilling at +2°C for 24 hr. The processing yield (%) was determined from the weights.

Chemical analysis

Representative samples from each treatment were homogenized and analyzed, prior to vacuum packaging (0 week), for percentage moisture, fat (ether—extractable), protein, ash, starch and sodium chloride according to standard AOAC (1984) procedures. Percent added water was also calculated according to AOAC (1984) formula. Sodium nitrite was determined by the ISO (1975) method. All analyses were performed in duplicate.

Purge loss

Two vacuum packages (≈ 250–300g each) per treatment were used to determine purge loss of frankfurters the 1st, 3rd and 5th week of storage in the dark at 4°C. Before packaging each link of frankfurters was dried with paper tissue and all links per package were weighed. After removing sausages from the package each link was again dried with paper tissue and all links per package were reweighed. Purge loss was determined from the difference in weights between the two measurements expressed as percentage of initial weight.

Color measurements

Color measurements were performed the 0 and 5th week of storage. A True—Color Neotec colorimeter was used to evaluate L, a and b (Hunter color system). The instrument was standardized using a white ceramic tile calibrated to tristimulus values of L = +96.0, a = −1.03, and b = +2.4. Two frankfurters per treatment were used. The surface of the glass tray was completely covered with sections of the frankfurters and four measurements were taken per link by rotating the glass tray one-quarter after each measurement. Data are means of eight measurements.

Rancidity determination

The 2-Thiobarbituric acid (TBA) test according to Tarladgis et al. (1960) was used to determine extent of oxidative rancidity after the 0, 1st, 3rd and 5th week. Two frankfurters were randomly sampled from each treatment. The frankfurters were ground in a chopper for 1 min and two 10-g portions were removed for TBA analysis. Duplicate determinations were conducted on each treatment. The amount of residual nitrite in each sample was taken into account and the amounts of sulfanilamide were added in the samples for TBA analysis according to the modifications of Shahidi at al. (1985). Readings were made on a LKB Ultrospec II spectrophotometer at 538 nm. The conversion factor 7.8 was used in calculation of TBA numbers.

Sensory evaluation

Sensory evaluation was conducted the 1st and 5th week of storage by a five-member trained panel. The panelists were chosen on the basis of previous experience in evaluating frankfurters. The following attributes were evaluated on a 5-point or 8-point scale: color (5 = very intensive, 1 = very poor), springiness (5 = extremely springy, 1 = not springy), firmness (8 = extremely firm, 1 = extremely soft), juiciness (8 = extremely juicy, 1 = extremely dry), flavor intensity (8 = extremely strong, 1 = extremely weak to unpleasant), overall palatability (8 = palatable, 1 = unpalatable). Each attribute was discussed and tests were initiated after panelists were familiarized with scales. Samples were prepared by steeping frankfurters in boiling water in individual pans 2 min. Warm, 2.5 cm long pieces from each treatment were randomly distributed for evaluation. Tap water was provided between samples to cleanse the palate.

Texture profile analysis

An Instron Universal Testing Machine, model 1140, was used to conduct texture profile analysis, as described by Bourne (1978), after 1 wk storage. Samples were prepared by steeping frankfurters in boiling water for 2 min and cooling to ambient temperature. Four 20 mm long sections per treatment were axially compressed by a two cycle compression test to 75% of original height. Force-time deformation curves were recorded at a crosshead speed 5 cm/min, chart speed 5 cm/min and full scale 50 kg. Texture variables of force and area measurements were: FP = force to fracture; F1 = maximum force for first compression; A1 = total energy for first compression; F2 = maximum force for second compression; A2 = total energy for second compression; springiness (S) = height sample recovered between end of first compression and start of second; gumminess = F1×A2/A1; chewiness = F1×A2/A1×S; and cohesiveness = A2/A1. Peak areas were determined by using the Lodd Graphic Data Analyzing System.

Skin strength

Skin strength of frankfurters was measured with a penetrometer Sur-Berlin, model PNR 6, equipped with a half-scale aluminum cone of 45 g and 20 g load weight. Samples were prepared by steeping frankfurters in boiling water for 2 min and cooling to ambient. The pointed part of the cone was placed at the surface of the frankfurters and the instrument was turned on for 10 sec to produce a puncture. The depth of puncture was measured in mm and higher depth means less skin strength. The same procedure was applied to five surface areas of each of two links of frankfurters per treatment. Data reported are means of ten measurements.

Statistical analysis

Data collected for batter characteristics, processing yield, chemical composition, sensory and instrumental texture profile values were analyzed by one-way analysis of variance. Data collected for purge losses, pH, TBA values and instrumental color were analyzed by a two factor factorial arrangement in a completely randomized design. The factors were: treatments (A,B,C,D) and storage time. Means were compared by using the $LSD_{0.01}$ test. Data analyses were performed using the MSTAT program.

RESULTS & DISCUSSION

MEAN pH and viscosity for uncooked batter of control and low-fat frankfurters containing olive oil were compared (Table 2). No differences ($P>0.05$) were found between pH of control and low-fat batters. The Brookfield viscosity of uncooked batter in low-fat frankfurters was higher ($P<0.05$) in treatments

Table 2—pH and viscosity for uncooked batter of control and low-fat frankfurters containing olive oil[f]

Parameters	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
pH	6.60 (0.26)[e]	6.51 (0.23)[e]	6.41 (0.12)[e]	6.33 (0.11)[e]
Brookfield viscosity (cp X 10[3])	414 (17.21)[e]	251 (14.93)[e]	339 (69.65)[ef]	456 (38.16)[f]

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for < 10% fat and 10%, 12% and 14% protein.
[e-f] Means within the same row with different superscript letters are different ($P < 0.05$).
[g] Means (standard deviation).

Table 3—Processing yield and proximate composition of control and low-fat frankfurters containing olive oil[g]

Parameters	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
Processing yield (%)	86.6 (3.8)[d]	80.2 (7.2)[e]	80.5 (5.9)[e]	80.5 (4.7)[e]
Moisture (%)	65.0 (0.8)[d]	70.8 (0.4)[e]	69.7 (0.5)[e]	68.0 (0.6)[f]
Protein (%)	10.9 (0.4)[d]	10.7 (0.1)[d]	12.4 (0.2)[e]	14.3 (0.2)[f]
Fat (%)	27.8 (0.7)[d]	11.6 (0.1)[e]	10.8 (0.4)[e]	10.8 (0.7)[e]
Ash (%)	2.8 (0.1)[d]	2.8 (0.1)[d]	2.7 (0.1)[de]	2.8 (0.1)[e]
Starch (%)	3.8 (0.4)[d]	4.3 (0.6)[d]	4.1 (0.8)[d]	4.1 (0.7)[d]
Sodium chloride (%)	1.8 (0.1)[d]	1.8 (0.1)[d]	1.8 (0.1)[d]	1.8 (0.1)[d]
Sodium nitrite (ppm)	112 (6.8)[d]	117 (7.5)[d]	125 (23.0)[d]	110 (13.0)[d]
Added water (%)[h]	12.8 (2.6)[d]	38.8 (0.5)[e]	24.9 (1.4)[f]	11.8 (0.8)[d]
Caloric content (Kcal/100g)[c]	312	163	168	172
Caloric content reduction (%)		47.6	46.1	44.7

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for < 10% fat and 10%, 12% and 14% protein.
[c] Calculations based on 9.1 Kcal/g for fat and 4.1 Kcal/g for protein and carbohydrates (Wirth, 1988).
[d-f] Means within same row with different superscript letters are different ($P < 0.05$).
[g] Means (standard deviation).
[h] Percent added water = (W − 4P)/(1 − 0.01W + 0.04P), where W = moisture %, P = protein % (AOAC, 1984).

with higher protein. No differences were found in viscosity between controls and low-fat treatments with 14% protein. The added water in both treatments was similar, 12.6% and 11.8% respectively (Table 3). These results agreed with Claus et al. (1989) who found that added water had greater effect than fat or protein on Brookfield viscosity.

Processing yields (Table 3) for control (86.6%) were 5.5–6.5% higher ($P<0.05$) than for low-fat treatments (80.2–80.5%). These results were in accordance with Townsend et al. (1971) who found that frankfurters with vegetable oil had lower processing yield than those prepared with animal fat. Preliminary experiments have shown that the small reduction of added salt in low-fat treatments, (16.1g/kg of batter instead of 17.5 g/kg in the control) had no effect on processing yield. Park et al. (1989) also reported that control frankfurters with 30% animal fat had 5–6% higher yield than low-fat treatments with ≈17% oil and the same added salt.

The proximate composition of control frankfurters was very near the targeted values. Total fat and protein concentrations of low-fat frankfurters were higher than targeted values, due to higher moisture loss during processing. For purposes of discussion, references to protein concentrations will be made according to formulated levels. The higher the protein content the lower the moisture content of the low-fat frankfurters except for the frankfurters with 10% and 12% protein where there was no difference ($P>0.05$). No differences ($P>0.05$) were found in sodium chloride and sodium nitrite content although added quantities in low-fat treatments were slightly different.

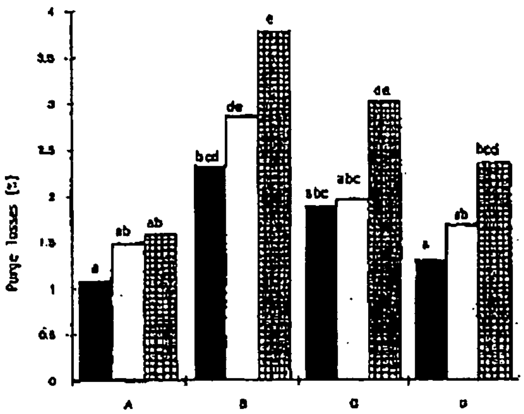

Fig. 1—Effect of storage time on purge losses of control (A) and low-fat frankfurters (B,C,D) containing olive oil. (A) Prepared with pork backfat and formulated for 28% fat and 11% protein. (B,C,D) Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein, respectively. a-e Bars with different superscript letters are different (P<0.05). ■ 1st wk, □ 3rd wk, ▦ 5th wk.

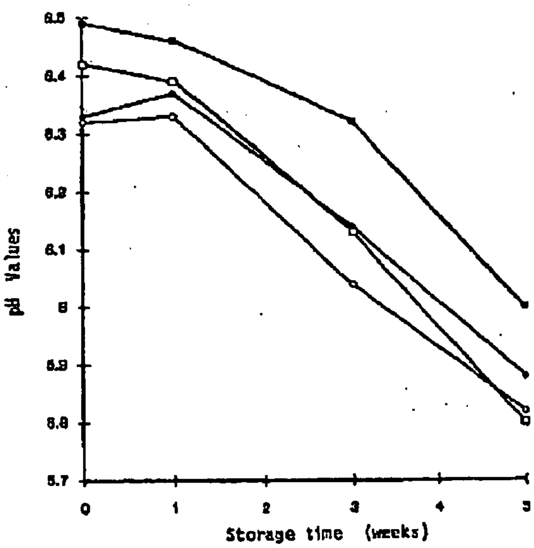

Fig. 2—pH values of control (A) and low fat frankfurters (B,C,D) containing olive oil. (A) ■——■ Prepared with pork backfat and formulated for 28% fat and 11% protein. (B) □——□, (C) ◆——◆, (D) ○——○ prepared with only virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein, respectively.

The total reduction in caloric content of low-fat frankfurters ranged from 44.7% to 47.6% compared to controls.

The low-fat treatment with 10% protein had higher ($P<0.05$) purge loss than all other treatments. Storage time had a significant effect on purge losses, especially in low-fat treatments (Fig. 1). The lower the protein level the higher the purge losses. The low-fat treatment with 14% protein was not different

Table 4—Effect of storage time on TBA values (mg malonaldehyde/kg) of control and low-fat frankfurters containing olive oil

Storage time at 4°C	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
0 week	0.81[d]	0.62[e]	0.69[d]	0.45[f]
1st week	0.84[d]	0.48[f]	0.55[e]	0.35[f]
3rd week	0.87[d]	0.97[d]	0.58[e]	0.62[e]
5th week	0.85[d]	0.63[e]	0.53[e]	0.42[f]

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein, respectively.
[d-f] Means within same row with different superscript letters are different (P < 0.05).

Table 5—Hunter color values of control and low-fat frankfurters containing olive oil

Hunter color numbers	Storage time (wk)	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
L (lightness)	0	55.0[e]	55.7[e]	54.4[e]	54.2[e]
	5	54.8[e]	55.7[e]	54.2[e]	53.8[e]
a (redness)	0	14.4[e]	11.1[f]	12.4[d]	14.7[e]
	5	13.6[e]	10.6[e]	11.8[d]	14.0[e]
b (yellowness)	0	12.9[e]	13.8[e]	13.2[e]	13.1[e]
	5	13.2[e]	13.9[e]	13.5[e]	13.1[e]

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein.
[c-e] Means within rows of same numbers with different superscript letters are different (P < 0.05).

Table 6—Sensory scores and instrumental texture profile of control and low-fat frankfurters containing olive oil[c]

Parameters	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
Sensory attribute:				
Color[g]	4.0[d]	3.0[e]	4.0[d]	4.5[d]
Springiness[h]	4.2[d]	4.1[d]	4.2[d]	4.3[d]
Firmness[i]	4.5[d]	2.7[e]	4.2[d]	5.5[f]
Juiciness[j]	7.2[d]	6.8[de]	6.4[de]	5.6[e]
Flavor intensity[k]	5.7[d]	5.6[d]	5.8[d]	5.8[d]
Overall palatability[l]	7.3[d]	5.7[e]	6.5[f]	6.4[f]
Skin strength (mm)	155.6[d]	168.0[d]	120.3[e]	77.0[f]
Texture profile:				
Fracturability (FF)[m]	34.0[d]	48.7[d]	61.1[e]	68.0[e]
1st bite hardness (F1)[m]	47.4[d]	43.6[d]	80.7[e]	109.2[f]
2nd bite hardness (P2)[m]	32.6[d]	24.8[d]	58.6[e]	87.5[f]
Springiness (S)[m]	15.1[d]	12.7[f]	15.4[de]	17.0[e]
Cohesiveness (A2/A1)	0.2[d]	0.1[d]	0.2[d]	0.2[d]
Gumminess (F1XA2/A1)	9.2[d]	6.7[d]	16.4[e]	23.7[f]
Chewiness (F1XA2/A1XS)	140.2[d]	87.6[d]	254.0[e]	403.6[f]

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for 10% fat and 10%, 12% and 14% protein.
[c] Data presented are means
[d-f] Means within row with different superscripts are different (P < 0.05).
[g] 5 = very intensive, 1 = very poor
[h] 8 = extremely springy, 1 = not springy
[i] 8 = extremely firm, 1 = extremely soft
[j] 8 = extremely juicy, 1 = extremely dry
[k] 8 = extremely strong, 1 = extremely weak to unpleasant
[l] 8 = palatable, 1 = unpalatable
[m] Expressed in Newtons

(P>0.05) in purge loss from the control during the storage period of 5 wk. Claus et al. (1990) found that the low-fat frankfurters had higher consumer shrink and purge losses. Higher purge losses of low-fat frankfurters were due to lower ionic strength. In our experiment the added salt in low-fat treatments was purposely reduced slightly. This probably contributed to further decrease of ionic strength in low-fat treatments. The increase in purge losses during storage was due to the decrease in pH. The correlation coefficient between purge losses and pH after the 1st week of storage was r = −0.644 (P<0.05). The pH of control was reduced from 6.5 to 6.0 and that of low-fat treatments from 6.4 to 5.8 during the 5 wk storage of vacuum-packed frankfurters at 4°C (Fig. 2). Paneras and Bloukas (1988) reported a decrease in pH from 6.3 to < 5.8 during the 9 wk storage of vacuum packed frankfurters at 3°C. Kempton and Bobier (1970) also found a decrease in pH from 6.3 to 5.4 during storage of frankfurters under vacuum at 5°C for 28 days. Simard et al. (1983) reported a decrease in pH from 6.18 to 5.42 during 7 wk storage of frankfurters under vacuum at 7°C. The pH decrease was attributed to activity of lactobacilli, and/or dissolution of CO_2 into meat tissue.

TBA values of refrigerated vacuum-packaged frankfurters over 5 wk were compared (Table 4). All low-fat treatments containing olive oil had lower (P<0.05) TBA values than control, initially and during 5 wk storage. The lower TBA values observed in olive oil containing frankfurters was attributed to tocopherols and phenolic substances with antioxidant activity in addition to nitrite. The TBA values of control treatment although higher than low-fat treatments were lower than acceptable range (<1.0) for oxidative rancidity (Ockerman, 1976). Storage time did not affect TBA values, probably due to the presence of curing ingredients, such as nitrite, phosphate and ascorbate, which also act as antioxidants.

Means for color measurements (Table 5) showed no difference (P>0.05) in Hunter L and b values between treatments and storage time. These results were in agreement with Ahmed et al. (1990) who found that decreasing fat content in fresh pork sausages with simultaneous increase in added water, did not affect Hunter L values. The lower the protein level of low-fat frankfurters the lower (P<0.05) the redness. The low-fat treatment with 14% protein level had the same (P<0.05) Hunter a value as the control. Differences in redness between low-fat treatments were due to different added water and protein levels. In low-fat treatments, added water increased from 12.4% to 39.2% while protein content was inversely reduced from 14.3% to 10.7% (Table 3). Reduced protein content resulted in dilution of myoglobin and consequently less red color. During the 5 wk refrigerated storage under vacuum no decreases in redness were observed.

Data on sensory scores and instrumental texture profiles of control and low-fat frankfurters containing olive oil were compared (Table 6). The low-fat treatment with 10% protein had lower (P<0.05) color, firmness and overall palatability scores. The treatment with 12% protein had similar (P>0.05) sensory attributes except palatability. The higher the protein content the higher (P<0.05) the firmness in low-fat frankfurters. Simon et al. (1965) and Claus et al. (1989) reported the same effects. Differences in flavor intensity between the control and low-fat treatments were not significant.

The 1st week of storage the control treatment had higher (P<0.05) overall palatability scores while differences between low-fat frankfurters with 12% and 14% protein were not significant. The frankfurters with 10% protein were very soft while those with 14% protein were harder and less juicy than the control. During the 5 wk cold storage a (P<0.05) reduction in overall palatability was found in all treatments (Fig. 3). The control treatment had higher (P<0.05) overall palatability while in low-fat treatments containing olive oil the higher the protein level the higher the overall palatability. The observed decrease in palatability during storage was probably due to microbial activity of lactic acid bacteria, which is in agreement with pH reduction (Fig. 2).

The control treatment had higher skin strength and fracturability and not significant changes in bite hardness, gumminess and chewiness with 10% protein low-fat frankfurters. This was probably due to the similar protein level of the 2 treatments

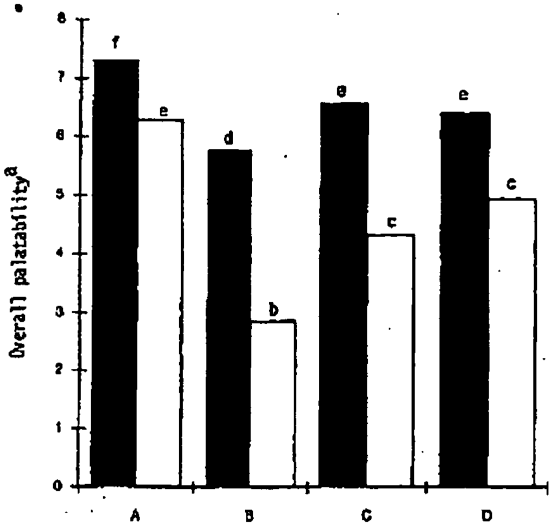

Fig. 3—Overall palatability scores the 1st and 5th week of storage of control (A) and low-fat frankfurters (B,C,D) containing olive oil. (A) Prepared with pork backfat and formulated for 28% fat and 11% protein (B,C,D) Prepared with virgin olive oil and formulated for < 10% fat and 10%, 12% and 14% protein, respectively. ■ 1st wk, □ 5th wk. a8 = palatable, 1 = unpalatable; b-e Bars with different superscript letters are different (P<0.05).

(Table 3). According to Saffle et al. (1964) the skin strength is developed by the migration of protein to the surface of frankfurters and subsequent denaturation during smoking. Differences between the control and low-fat treatments with 12% and 14% protein for skin strength, fracturability, 1st and 2nd bite hardness, springiness, gumminess and chewiness were significant. The higher the protein in low-fat treatments the higher ($P<0.05$) was the skin strength, the 1st and 2nd bite hardness, gumminess and chewiness. Low-fat treatments with 12% and 14% protein had no significant differences for fracturability and springiness while all treatments had the same ($P<0.05$) cohesiveness.

CONCLUSIONS

LOW-FAT FRANKFURTERS (10% fat) could be manufactured with olive oil and without added animal fat. The low-fat frankfurters would be highly desirable from a diet/health standpoint as they contain monounsaturated vegetable oil, have lower caloric value, reduced cholesterol and a higher protein content. Among low-fat treatments with olive oil, that with ≈ 12% protein had quality characteristics most comparable to the control.

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